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**TITLE:** *Subcortical brain volume differences of participants with ADHD across the lifespan: an ENIGMA collaboration*

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**ABSTRACT**

**BACKGROUND** Neuroimaging studies show structural alterations in several brain regions in children and adults with attention-deficit/hyperactivity disorder (ADHD). Through the formation of the worldwide ENIGMA ADHD Working Group, we addressed weaknesses of prior imaging studies and meta-analyses in sample size and methodological heterogeneity.

**METHODS** Our sample comprised 1713 participants with ADHD and 1529 controls from 23 sites (age range: 4-63 years; 66% males). Individual sites analyzed magnetic resonance imaging brain scans with harmonized protocols. Case-control differences in subcortical structures and intracranial volume (ICV) were assessed through mega- and meta-analysis.

**FINDINGS** The volumes of the accumbens (Cohen's *d*=-0·15), amygdala (*d*=-0·19), caudate (*d*=-0·11), hippocampus (*d*=-0·11), putamen (*d*=-0·14), and ICV (*d*=-0·10) were found to be smaller in cases relative to controls. Effect sizes were highest in children, case-control differences were not present in adults. Explorative lifespan modeling suggested a delay of maturation and a delay of degeneration. Psychostimulant medication use or presence of comorbid psychiatric disorders did not influence results, nor did symptom scores correlate with brain volume.

**INTERPRETATION** Using the largest data set to date, we extend the brain maturation delay theory for ADHD to include subcortical structures and refute medication effects on brain volume suggested by earlier meta-analyses. We add new knowledge about bilateral amygdala, accumbens, and hippocampus reductions in ADHD, and provide unprecedented precision in effect size estimates. Lifespan analyses suggest that, in the absence of well-powered longitudinal studies, the ENIGMA cross-sectional sample across six decades of life provides a means to generate hypotheses about lifespan trajectories in brain phenotypes.

**FUNDING** National Institutes of Health

**KEYWORDS:** ADHD, Subcortical brain volumes, imaging, lifespan, meta-analysis, amygdala

**Research in context**

**Evidence before this study.** After searching for all prior meta-analysis performed on brain volume differences in ADHD including the subcortical regions until the 1st of February 2015 using the search terms 'ADHD', 'structural', 'brain', and 'meta-analysis [Title]' and 'english' [Language] in Pubmed, we found four published meta-analyses. The largest of those meta-analysed data on 565 cases and 583 controls (children only). The published meta-analyses had three major limitations: 1. Power was only sufficient to detect effect sizes of Cohen's *d* of 0.15 and higher, which we know to be insufficient based on results in other psychiatric disorders. 2. Existing studies only used published data as source material, which limited their ability to address covariates that may vary among studies, like age, and medication. 3. The existing meta-analyses included studies using different segmentation software and quality control procedures, a limitation contributing to heterogeneity across samples.

**Added value of this study.** The current multi-site study, with data on 1713 cases and 1529 controls included, is by far the largest and best-powered study to date on brain volumes in ADHD. Data of all sites were newly analyzed using harmonized methods. Our work implicates new structures, amygdala and hippocampus, in ADHD, and provides unprecedented precision in effect size estimates. Our results, covering most part of the lifespan, showed most pronounced effects in childhood.

**Implications of all the available evidence.** We confirm, with high powered analysis, that ADHD patients truly have altered brains, i.e. that ADHD is a disorder of the brain. This is a clear message for clinicians to convey to parents and patients, which can help to reduce the stigma of ADHD and get a better understanding of ADHD. This way, it will become just as apparent as for major depressive disorder, for example, that we label ADHD as a brain disorder. Also, finding the most pronounced effects in childhood provides a relevant model of ADHD as a disorder of brain maturation delay.

Finding the biggest effect in the amygdala is another important message, as it links ADHD to emotional regulation problems. Those are frequently found in patients with ADHD, but these disease characteristics

have not (yet) made it into the official DSM-criteria. Our work shows neurobiological support for the inclusion of this domain in the core ADHD phenotype.

## INTRODUCTION

Attention-deficit/hyperactivity disorder (ADHD) is a common neuropsychiatric disorder with a prevalence of 5.3% in childhood[1]. Two-thirds of patients with an ADHD diagnosis in childhood continue to have persistent, impairing symptoms in adulthood[2]. ADHD is characterized by age-inappropriate symptoms of inattention and/or hyperactivity and impulsivity[3]. Many imaging studies, often in small samples, have reported brain structural and functional differences between individuals with ADHD and controls, both in childhood and adulthood. Five meta-analyses of structural neuroimaging studies in ADHD have been published (**Table 1**). The first meta-analysis pooled region of interest brain volumes studies[4], while the others pooled voxel-based morphometry (VBM) studies[5-8]. Most consistent results across studies were for reduced volumes of (parts of) the basal ganglia for patients compared with healthy controls. Two meta-analyses showed that, with increasing age, basal ganglia structural differences between cases and controls tended to decrease, and that stimulant treatment was associated with normalization of these brain structures[5,6].

Brain volumes have also been associated with clinical features of ADHD; smaller volumes of caudate, cerebellum, and frontal and temporal gray matter have been associated with greater symptom severity[9]. Also in the general population, ADHD symptoms correlated with volumetric brain measures[10,11].

Identifying structural brain differences in people with ADHD is important to further our insights into the nature of ADHD. So far, analyses of brain structures in ADHD have been limited in size and statistical power (**Table 1**); the sample size of the largest published meta-analysis on brain volume (n=565 cases and n=583 controls) allowed the identification of differences in brain volume with Cohen's *d* effect sizes of ≥0·15 with 80% power (G*Power[12]). Analyses of other psychiatric disorders show that smaller effects are likely[13]. Existing meta-analyses for ADHD only used published data as source material, which limited their ability to address covariates that may vary among studies, like age and medication[5,6]. In addition, the

existing meta-analyses included studies using variable methods and protocols such as the segmentation software and quality control.

To overcome such issues and perform collaborative studies of maximal power, we founded the ENIGMA ADHD Working Group. This worldwide collaboration enabled analyses of existing individual data, improving upon earlier meta-analyses by basing analyses on the use of harmonized segmentation and quality control protocols. Our increased sample size compared to all earlier studies supported both mega- and meta-analysis (**sMethods, appendix**) designs across 60 years of the lifespan. We selected subcortical brain volumes as our target, because of neurodevelopmental theories hypothesizing ADHD to be linked to early-emerging, persistent subcortical abnormalities[14] and building on the results of earlier meta-analyses, which showed that deviations in these volumes were most consistently observed. In addition, we investigated intracranial volume (ICV) as a measure of total brain volume. Analyzing data from 23 cohorts with a sample size of n=3200 enabled us to detect the case-control effect sizes observed in other psychiatric disorders. In addition, the mega-analysis design also allowed investigation of associations with symptom scores, age, psychostimulant medication use, and comorbidity with other psychiatric disorders.

## MATERIALS AND METHODS

*Contributing studies*

The ENIGMA ADHD Working Group was formed in 2013 to aggregate structural magnetic resonance imaging (MRI) data from participants with ADHD and healthy controls across the lifespan. Details about the diagnostic procedures for each site are listed in the **appendix (sTable1)**. The group adopted a rolling inclusion design, in which new groups can join at any time, but data-freezes allow analysis at fixed time points. The data-freeze for the current subcortical analysis was set at February 8, 2015. The analyzed

sample comprised 23 cohorts, for details see **Table 2.** Each participating site had approval from its local ethics committee to perform the study and to share de-identified, anonymized individual data.

*Neuroimaging*

Structural T1-weighted brain MRI data were acquired and processed at the individual sites. The images were analyzed using standardized protocols to harmonize analysis and quality control processes (**sMethods, appendix,** and http://enigma.ini.usc.edu/protocols/imaging-protocols/). Fully-automated and validated neuroimaging segmentation algorithms based on FreeSurfer versions 5.1 or 5.3 were used (**sTable1, appendix)**. To make sure no effects of FreeSurfer version influenced the results[13], we performed an additional analysis, adding version number as a covariate to our main model (see below). For each participant, we computed ICV and left and right volumes of the accumbens, putamen, pallidum, caudate, thalamus, amygdala, and hippocampus. For further analysis, we used the mean of the left and right volume ((R+L)/2). For an overview of single site subcortical structures, see appendix (**sFigure1)**. Outliers were determined at above and below 1.5-times the interquartile range per cohort and group (case/control) and were excluded (**sFigure1, appendix)**[15].

*Case-control differences of subcortical brain volumes and ICV*

By pooling all available individual data from all cohorts, a mega-analysis (for explanation see the **sMethods, appendix**), we investigated the differences between cases and controls on subcortical volumes and ICV. After excluding collinearity of age, sex, and intracranial volume (ICV) (variance inflation factor <1.2) and normality testing, the mega-analysis of each subcortical volume was performed using a linear mixed model (lme) by running the package nlme in R (version3.1-117). The model included *diagnosis* (case=1 and control=0) as factor of interest, *age*, *sex*, and *ICV* as fixed factors, and *site* as

random factor. In the analysis of ICV, ICV was omitted as covariate from the model. Handedness was added to the model to correct for possible effects of lateralization, but was excluded from the model when there was no significant contribution of this factor. To calculate Cohen's *d* effect size estimates, adjusted for age, sex, site, and ICV, we used the *t*-statistic from the factor d*iagnosis* in the model. In a post-hoc analysis, left and right volumes were studied separately.

To make sure that no unobserved factor biased our analysis of case-control differences, meta-analysis was also performed by linear regression analysis for each volume and for each sample separately, taking age, sex, and ICV into account. The R-package "metaphor" (version 1.9-1[16]) was used to perform an inverse variance-weighted, random-effects meta-analysis, in accordance with other ENIGMA Working Groups[13,15] (**sMethods, appendix**).

*Effects of age*

The effect of age on subcortical volume and ICV was studied by running the above described model for groups stratified by age: in children aged 14 or younger, adolescents aged 15 until 21 years of age, and in adults, aged 22 and older. We removed samples that were left with 10 subjects or less due to the stratification. As it is likely that the effects of age do not strictly follow a linear model, we only report linear effects of age and the effect of age*diagnosis for the sake of being complete. In addition, more explorative modeling was done to better understand the effects of age, by plotting moving averages and using fractional polynomials to fit non-linear models to the data (**sMethods, appendix**).

*Significance threshold*

Multiple comparisons correction for 32 tests (8 volumes and 4 groups: all, children, adolescents, and adults) was applied by using a false discovery rate with q=0·05 resulting in a *p*-value significance threshold of *p*=0·156.

*Exploration of effects of sex, psychostimulant medication, and clinical measures*

To explore the effects of sex on brain volume, the results of the term *sex* from the main model are reported. To examine associations between prior psychostimulant treatment and regional brain volume, the mega-analysis model was run again, including only patients with medication information available (**sTable1, appendix**). To test, whether acute effects of psychostimulant medication confounded possible brain volume differences between participants with ADHD and healthy controls, we excluded subjects treated with stimulants at the time of their participation in the study (participants receiving other types of treatment were retained). In addition, as previous meta-analyses had found an association between stimulants and brain volumes[5,6], we compared patients, who had ever used stimulant medication, to patients, who were lifetime stimulant-naïve. We explored the effects of ADHD symptom scores and presence/absence of co-morbid disorders on those brain volumes that differed significantly between participants with ADHD and healthy controls, for details see appendix (**sMethods and sTable2, appendix)**.

*Role of the funding sources*

The funders had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all of the data and the final responsibility to submit for publication.

**RESULTS**

We included data from 1713 participants with ADHD and 1529 healthy controls (**Table 2**) with a median age of 14·0 (range 4-63) years.

*Case-control differences in subcortical volumes and ICV*

As shown in **Table 3**, the mega-analysis indicated that participants with ADHD had significantly smaller volumes for the accumbens, amygdala, caudate, hippocampus, putamen, and ICV. *Post-hoc* analyses for the subcortical regions showed these effects to be bilateral (**sTable3, appendix**). No effect of FreeSurfer version of handedness was found **(sTable4&5, appendix)**.

Results of the case-control meta-analysis were largely comparable to those of the mega-analysis, but volume differences for accumbens and hippocampus were not significant (**sTable6, appendix)**. Heterogeneity ($I^2$) across samples was low to moderate; heterogeneity was highest for hippocampus (**sTable6, appendix**) and might be indicative of non-linear effects of site for this structure.

*Effect of age*

Age-stratified analyses revealed significant case-control differences in children for the accumbens, amygdala, caudate, hippocampus, putamen, and ICV (**Table 4 and Figure 1**). Effect sizes were higher than those for the entire sample. In the adolescent group, there was a significant case-control difference in the hippocampus. In adults, none of the case-control comparisons remained significant. Figure 1 suggested an interaction effect for age-group and diagnosis on hippocampus volume; this was nominally supported by linear interaction statistics ($p$=0.03; **sTable7, appendix**). Explorative modeling using moving averages (**Figure 2**) also showed the age effects to cluster early in life, with higher age of attaining peak volumes in the ADHD group. The moving averages also hinted at potential later onset of volume decrease in the ADHD group, most clearly seen in accumbens and putamen. Sample sizes after age 50

years were limited (**sFigure3, appendix**), and resulted in wider confidence intervals in the moving average analyses. The fractional polynomial analyses also supported different developmental models for ADHD cases and controls for amygdala, hippocampus, putamen, thalamus, and ICV (**sFigure4 & sTable8, appendix**).

*Effect of sex*

Consistent with literature documenting smaller brains in females[17], all but two subcortical structures, accumbens and caudate, showed main effects of sex in the mega-analysis (**Table 3**). None of the volumes showed differential sex effects for participants with ADHD and controls.

*Effect of medication*

Information on current medication use was available for 1254 participants with ADHD; 455 participants with ADHD were on psychostimulant medication (methylphenidate or amphetamine) at the time of scanning, with over half of the studies using a washout period of 24/48 hours (**sTable1, appendix**); 799 participants with ADHD were not taking stimulant medication at scan time. Case-control differences in brain volumes after excluding participants currently on stimulant medication (**Table 5**) were comparable in effect sizes to those observed in the main analysis.

For 719 participants with ADHD, information was available on lifetime usage of stimulant medication. Of these, 82 participants (11%) had never taken stimulant medication, compared to 637 patients, who used stimulant medication somewhere in their lifetime for a period of more than 4 weeks. No differences in any of the volumes were found by directly comparing these two groups.

*Association of clinical measures with subcortical brain volumes and ICV*

Meta-analysis of the correlation between ADHD symptom scores in cases and brain volumes revealed no significant effects; only a nominally significant effect ($p$=0.02) was observed for caudate volume (**sTable9 & sFigure6, appendix**). Neither were there any significant correlations when only the childhood samples were entered in the meta-analysis. Also, the observed case-control brain volume differences were not explained by the presence of another comorbid psychiatric disorder (**sTable10, appendix**).

## DISCUSSION

Here, we report the largest study to date of brain volume differences between participants with ADHD and healthy individuals. Through worldwide collaboration in the ENIGMA ADHD Working Group, data on 1713 participants with ADHD and 1529 healthy controls were newly analyzed, using harmonized quality control and segmentation procedures. Compared to previous meta-analyses, our study newly identified amygdala, accumbens, and hippocampus volumes to be smaller in participants with ADHD, and extended earlier findings for reduced caudate and putamen volumes by showing those to be bilateral rather than unilateral[5,7]. Significant volume differences had small effect sizes (ranging from $d$=-0·10 to $d$=-0·19). Meta-analysis confirmed these results. Age-stratification showed volume differences to cluster in childhood, no differences were seen in adulthood. The volume differences were equally apparent in those treated with psychostimulant medication and those naïve to psychostimulants. Finally, no correlations with quantitative scores of ADHD symptoms were found in cases, nor did comorbidity with other psychiatric disorders explain the findings.

The work presented here carries several important messages for the clinical field. First, our results coming from highly powered analysis, confirm that ADHD patients truly have altered brains, i.e. that ADHD is a disorder of the brain. This is a clear message for clinicians to convey to parents and patients, which can help to reduce the stigma that ADHD is just a label for difficult kids, and caused by incompetent parenting. We hope this work will contribute to a better understanding of ADHD in the general public, and

that it becomes just as apparent as for major depressive disorder, for example, that we label ADHD as a brain disorder. Second, finding the most pronounced effects in childhood and showing delayed peaks of subcortical volume maturation provides a relevant model of ADHD as a disorder of brain maturation delay. Third, the brain differences we have found are not caused by any co-morbid disorders, medication effects, or ADHD symptom severity, but are exclusively related to the ADHD diagnosis. Lastly, finding the largest effect in the amygdala is another important message, as it links ADHD to emotional regulation problems. Those are often present in patients with ADHD, but these disease characteristics have not (yet) been included into the official DSM-criteria. Our work shows neurobiological support for the inclusion of this domain in the core ADHD phenotype, asking for more acknowledgement of the importance of emotion regulation problems in the ADHD patient.

Our findings for striatum volume reduction are in line with current models of ADHD[18]. Differences in caudate volume are the most consistent finding for ADHD[4-6], and also smaller putamen volumes have been frequently reported[5-7]. Our study now provides robust effect size estimates for those structural differences and shows that effects are bilateral. Although identified before in a single study[19], our findings extend the meta-analytic literature to the third striatal volume, nucleus accumbens. Novel meta-analytic findings of our study are for amygdala and hippocampus. Previous work in single studies had found effects in these structures[20-22], but also failed to replicate in others e.g.[23,24]. For amygdala volume, which showed the largest effect size in our study (*d*=-0·19; *d*=-0·18 in children), and for accumbens, the lack of earlier meta-analytic evidence for its role in ADHD might be due to the fact that these are small structures, for which automatic segmentation performs less well[25]. A more highly powered analysis may therefore have been necessary to overcome the experimental inaccuracy of these measures. Prior work provides functional evidence for a role of amygdala, accumbens, and hippocampus in ADHD. Dysfunction of the amygdala is associated with difficulties recognizing emotional stimuli, callous unemotional traits, and with emotion regulation in general[26,27]. Difficulties in recognizing emotional stimuli, diminished emotional

reactions to pleasant stimuli, and higher levels of callous unemotional traits have all been linked to ADHD[28-31], and amygdala volume has been associated with hyperactivity[20]. The accumbens, with its prominent role in reward processing, is central to motivational and emotional dysfunction in ADHD[18]. The results of the hippocampus are less straight-forward, as there is not so much evidence for a deficit in long-term memory in ADHD patients the hippocampus' main function[32]. However, there are also reports on the hippocampus playing a role in the regulation of motivation and emotion, which is impaired in ADHD[33].

Importantly, effect sizes observed in our study were similar to those found for other psychiatric disorders analysed using the ENIGMA procedures, in particular major depression and bipolar disorder [13,34]. The scale of the effects is consistent with expectations for a heterogeneous disorder like ADHD. The specific pattern of findings may partially differentiate ADHD from the other psychiatric disorders analysed using similar procedures, i.e. schizophrenia, bipolar disorder, and major depressive disorder[13,15,34]. Especially effects on caudate and putamen seem to be ADHD-specific among the four. However, as mostly adults were investigated for the other three disorders, formal analyses taking age into account will need to be performed to make valid statements.

The results of the age-stratified analysis indicate that subcortical volume differences in ADHD are most prominent in children, and non-existent in adults. Our additional exploratory models suggest that this is not the entire story on age effects, though care in interpreting this result is needed because of the cross-sectional design of this study. Based on our findings across different approaches, we propose a model of altered trajectories of subcortical volume in ADHD. Our data suggest a delayed peak volume in participants with ADHD, which is reminiscent of earlier reports of altered velocity of cortical development in a longitudinal sample[35]. This model should be confirmed by longitudinal analyses, especially since the childhood and adult ADHD samples included in this study represent different subgroups of the population: childhood ADHD samples include those who will later remit and those who will persist having ADHD in adulthood, the adult ADHD samples include only the latter. In addition to the delays in subcortical brain

maturation at early age, our exploratory work also tentatively suggest later onset of decreases in subcortical volumes beyond the 4th decade of life in ADHD. However, since sample sizes in our analysis dropped dramatically above age 25 years, and we had insufficient data to study age effects after 60 years, this work is still hampered by not having sufficient subjects per site to rule out site-biases in those age ranges. As long as ADHD in old age is still a blind spot in ADHD research, it will be difficult to test the validity of such findings.

Prior meta-analyses found associations between the percentage of treated patients and right caudate and amygdala/uncus volumes[5,6]. In our analysis, in which we were able to directly compare treated to non-treated participants with ADHD in a sample exceeding the size included in the two previous meta-analyses 4-fold, we did not confirm such associations with brain volume. This is in line with the most recent meta-analysis[8]. However, since our study had a non-randomized, cross-sectional design, some caution to interpreting these results is warranted, as the design of this study was not optimal to test medication effects. Also, as both prior meta-analyses used voxel-wise maps, there is a possibility that the observed normalizing effects of medication were too local to be picked up by volumetry.

We did not observe associations of brain volumes with clinical measures, i.e. comorbidity or ADHD symptom scores. The absence of an association with comorbidity suggests that the brain volume reductions are robustly linked to ADHD itself, rather than being a secondary phenomenon caused by comorbidity. The absence of significant associations between brain volumes and symptom ratings is not surprising, given that brain function is based on distributed networks of brain regions rather than individual brain regions[36]. Still, previous studies did find single volume-function associations[9,37], which we do not replicate here. We also could not replicate an earlier reported (modest) correlation of a total brain volume measure highly related to ICV with ADHD symptom severity in a similarly sized population sample[10]. In addition to the above, not finding effects of symptom scores might also be due to the heterogeneity of the instruments used by different cohorts in our study and/or differences in raters

(clinicians, teachers, parents). In addition, the sample size was halved in this case-only analysis, and the distribution of scores was skewed to the clinical range. In line with models of fronto-striatal dysfunction in ADHD, one could hypothesize that cortical structures might play a more important role in the severity of symptoms in ADHD patients than the subcortical structures[14].

This study has several strengths and limitations. A clear strength is the sample size, being the largest mega-/meta-analysis to date, with enough power to detect effects as small as *d*=0.08. Another strength is the harmonization of segmentation protocols across all contribution sites, reducing imprecision caused by differences in methods. Nonetheless, diagnostic routines and acquisition of imaging data still differed between sites, a limitation contributing to heterogeneity across samples. A strength was also the opportunity for mega-analysis. While effect sizes were similar to the meta-analysis, the mega-analysis allowed a more powerful detection of case-control volume differences. Mega-analysis also enabled effects of age, sex, comorbidity, and medication to be studied, although accounting for site in these analyses might have somewhat masked age effects (as many studies had a restricted age range). Modeling age in a cross-sectional study is challenging but we have used several approaches to understand the effects of age, however, we should be cautious and interpret our findings as hypothesis-generating for future studies.

To conclude, this first result of our world-wide collaboration confirms and extends previous findings of reduced striatal volume in ADHD. Optimizing sample size and harmonizing methods across studies allowed us to identify additional differences in amygdala and hippocampal volumes potentially contributing to problems in emotion regulation, motivation, and memory in ADHD. Brain volume differences were most prominent in children. We invite interested researchers to join the next studies of the ENIGMA ADHD Working Group. In this way, we may optimally benefit from efforts already invested in individual studies to better understand this common yet still vexing disorder.

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*Protocol design, quality testing, and analysis:* Hoogman, Bralten, Hibar, Mennes, Zwiers, Schweren, Hulzen, Medland, Shumskaya, Jahanshad, Faraone, Thompson, Franke

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**FIGURE LEGENDS**

**FIGURE 1.** Displayed are the Cohens *d* effect sizes of differences between patients with ADHD and healthy controlsfor subcortical volumes and ICV, for 4 separate groups:1.) all subjects, 2.) children only (<15years), 3.) adolescents only (15-21 years), and 4.) adults only (>21 years). *significant after false discovery rate (FDR) correction; †nominal significant at p<0.05

**FIGURE 2.** Displayed are the moving averages, corrected for age, sex, ICV and site for the subcortical volumes.

**TABLES and FIGURES**

**Table 1.** Overview of published structural neuroimaging meta-analyses in ADHD.

| Study | Subjects | Sample size (case/controls) | Image analysis method | Main results |
|---|---|---|---|---|
| **Valera *et al.*, 2007** | Children only | 565/583 | Brain volumetry | Reduced volume of splenium of the corpus callosum, cerebral volume, and right caudate nucleus in patients. |
| **Ellison-Wright *et al.*, 2008** | Children only | 114/143 | VBM | Smaller right putamen / pallidum region in patients. |
| **Nakao *et al.*, 2011** | Children and adults | 378/344 | VBM | Smaller basal ganglia in patients. Increasing age and long-term medication use associated with reduced case-control differences. |
| **Frodl *et al.*, 2012** | Children and adults | 320/288 | VBM | Right globus pallidus, right putamen, and caudate are reduced in patients. Increasing age and treatment tended to be associated with reduced deficits in patients. |
| **Norman *et al*, 2016** | Children and adults | 931/822 | VBM | Decreased grey matter volume in right basal ganglia, insula, ventromedial orbitofrontal cortex, medial prefrontal cortex, right anterior cingulate cortex. No association between the grey matter abnormalities and long-term stimulant use |

VBM = voxel-based morphometry

**Table 2.** Overview of cohort characteristics. For a more detailed description and references for the assessments and neuroimaging procedures, see sTable 1 in the appendix.

| Sample name | Site, country of origin | N Total | N Cases (M/F) | N Controls (M/F) | Age ± SD |
|---|---|---|---|---|---|
| ADHD-WUE | Würzburg, GER | 118 | 32/30 | 26/30 | 39.68±11.44 |
| ADHD-DUB1 | Dublin, IRL | 75 | 27/9 | 31/8 | 22.29±5.23 |
| ADHD-DUB2 | Dublin, IRL | 20 | 16/4 | - | 33.65±10.15 |
| ADHD-Mattos | Rio de Janeiro, BRA | 17 | 10/7 | - | 22.94±1.39 |
| ADHD200-KKI | Baltimore, USA | 94 | 15/10 | 41/28 | 10.22±1.34 |
| ADHD200-NYU* | New York, USA | 260 | 115/36 | 54/55 | 11.47±2.92 |
| ADHD200-Peking | Peking, CHN | 245 | 90/12 | 84/59 | 11.70±1.96 |
| ADHD200-OHSU | Oregon, USA | 109 | 29/13 | 30/37 | 9.13±1.25 |
| ADHD-UKA | Aachen, GER | 181 | 95/7 | 53/26 | 11.21±2.68 |
| Bergen-adultADHD | Bergen, NOR | 81 | 21/17 | 16/27 | 31.21±6.74 |
| Bergen-SVG | Bergen, NOR | 54 | 20/5 | 20/9 | 10.05±1.20 |
| DAT-London | London, GBR | 56 | 27/0 | 29/0 | 15.78±2.10 |
| IMpACT-NL | Nijmegen, NLD | 245 | 49/76 | 49/71 | 35.49±11.39 |
| MGH-ADHD | New York, USA | 148 | 42/37 | 29/40 | 35.76±12.03 |
| NICHE | Utrecht, NLD | 158 | 68/10 | 67/13 | 10.42±1.95 |
| NYU ADHD | New York, USA | 80 | 22/18 | 22/18 | 31.58±9.44 |
| UAB-ADHD | Barcelona, SPA | 198 | 82/21 | 64/31 | 25.80±13.02 |
| ZI-CAPS | Mannheim, GER | 35 | 17/5 | 7/6 | 12.73±1.23 |
| ADHD-Rubia | London, GBR | 77 | 44/0 | 33/0 | 13.95±2.19 |
| NeuroImage-ADAM | Amsterdam, NLD | 182 | 73/24 | 57/28 | 17.16±3.19 |
| NeuroImage-NIJM | Nijmegen, NLD | 178 | 89/50 | 23/16 | 16.89±3.41 |
| NIH | Bethesda, USA | 502 | 168/83 | 168/83 | 9.97±3.09 |
| MTA | Irvine, USA | 129 | 73/15 | 31/10 | 24.6±1.4 |
| **Total** | | **3242** | **1713** | **1529** | **18.6±11.81** |

*One subject was excluded because of missing gender status

**Table 3.** Results of the mega-analysis of subcortical brain volumes in the total sample.

| | **N Cases/ Controls** | **Adjusted mean volume estimate (SEM)[1] Cases/Controls** | **p-value for *Diagnosis*** | **Cohen's *d (95%CI)*** | **Other significant terms in the model** |
|---|---|---|---|---|---|
| Accumbens | 1652/ 1471 | 656·5 (1·2) / 673·7(2·1) | **4·98x10$^{-9}$** | -0·15 (-0.22- -0.08) | ICV, Site, Age |
| Amygdala | 1598/1463 | 1554·1 (3·7) / 1577·8(3·7) | **3·69x10$^{-7}$** | -0·19 (-0.26 - -0.11) | Sex, ICV, Site |
| Caudate | 1659/1489 | 3927·8 (8·2) / 3964·6(8·6) | **0·001** | -0·11 (-0.18 - -0.03) | ICV, Site, Age |
| Hippocampus | 1599/1436 | 4147·8 (8·1) / 4163·7(8·5) | **0·004** | -0·11 (-0.18 - -0.03) | Sex, ICV, Site |
| Pallidum | 1651/1471 | 1764·8 (4·6) / 1763·7(4·9) | 0·95 | -0·00 (-0.07 - 0) | Sex, ICV, Site, Age |
| Putamen | 1660/1497 | 6025·7 (13·7) / 6100·6(14·6) | **6·36x10$^{-9}$** | -0·14 (-0.21 - -0.07) | Sex, ICV, Site, Age |
| Thalamus[#] | 1405/1242 | 7683·3 (17·8) / 7611·6(18·5) | 0·39 | -0·03 (0·03 - -0·10) | Sex, ICV, Site, Age |
| ICV | 1693/1513 | 1513597·3 (2741·3) / 1501680·7(2924·7) | **0·006** | -0·10 (0·04 - -0·16) | Sex, Site, Age |

**Bold** p-values are significant at the FDR-corrected threshold of *p*=0·0156, *italic* p-values nominally significant at p<0·05 [#]thalamus volume was not available from the NIH sample.[1]Adjusted mean volume estimate and standard error of the mean, corrected for age, sex, ICV, and site.

**Table 4.** Results of the mega-analysis of subcortical brain volumes in the stratified age groups

| | **Children (<15)** | | | **Adolescents (15-21)** | | | **Adults (21>)** | | |
|---|---|---|---|---|---|---|---|---|---|
| | **N Cases/ Controls** | **p-value for *Diagnosis*** | **Cohen's *d* *(95%CI)*** | **N Cases/ Controls†** | **p-value for *Diagnosis*** | **Cohen's *d* *(95%CI)*** | **N Cases/ Controls** | **p-value for *Diagnosis*** | **Cohen's *d* *(95%CI)*** |
| Accumbens | 810/827 | **0·0001** | -0·19 (-0·29 - 0·10) | 323/224 | 0·61 | -0·04 (-0·22 - 0·12) | 510/415 | 0·12 | -0·10 (-0·23 - -0·03) |
| Amygdala | 767/820 | **0·0003** | -0·18 (-0·28 - -0·08) | 321/226 | 0·12 | -0·14 (-0·31 - 0·03) | 500/412 | *0·03* | -0·14 (-0·27 - -0·01) |
| Caudate | 825/840 | **0·006** | -0·13 (-0·23- -0·04) | 324/224 | 0·28 | -0·10 (-0·27 - 0·07) | 502/420 | 0·30 | -0·07 (-0·20 - 0·05) |
| Hippocampus | 764/802 | **0·012** | -0·12 (-0·22 - -0·03) | 320/225 | **0·006** | -0·24 (-0·42 - -0·08) | 506/404 | 0·38 | 0·06 (-0·07 - 0·19) |
| Pallidum | 816/831 | 0·79 | -0·01 (-0·11 - 0·08) | 321/223 | 0·78 | 0·02 (-0·15 - 0·20) | 506/412 | 0·51 | 0·04 (-0·08 - 0·17) |
| Putamen | 836/854 | **0·0002** | -0·18 (-0·28 - -0·09) | 329/228 | 0·83 | -0·02 (-0·19 - 0·15) | 499/416 | 0·23 | -0·08 (-0·21- 0·05) |
| Thalamus[#] | 604/616 | 0·89 | 0·01 (0·06 - -0·10) | 288/202 | 0·74 | 0·03 (-0·15 - 0·21) | 503/416 | 0·28 | -0·07 (-0·20 - -0·06) |
| ICV | 837/854 | **0·003** | -0·14 (0·04 - -0·24) | 330/229 | 0·13 | -0·13 (-0·30 - 0·04) | 515/422 | 0·91 | 0·01 (0·06 - -0·12) |

**Bold** p-values are significant at the FDR-corrected threshold of *p*=0·0156, *italic* p-values nominally significant at p<0·05 [#]thalamus volume was not available from the NIH sample. † Due to a sample size lower than 10, the data for the following cohorts in analysis of the adolescent group were omitted: ADHD-Mattos (n=2), ADHD-WUE (n=2), BergenAdultADHD (n=4), MTA (n=2), Niche (n=7), ZI-CAPS (n=2).

**Table 5.** Results of the exploration of the effect of medication on case-control differences

| | **Patients currently not taking stimulants versus controls*** | | | **Stimulant use in patients: positive versus negative lifetime history** | |
|---|---|---|---|---|---|
| | **n Cases/ Controls** | **Cohen's *d (95%CI)*** | **p-value for *Diagnosis*** | **n Never / ever stimulant use in patients only** | **p-value for positive versus negative for lifetime stimulant use** |
| Accumbens | 776/1484 | -0·12 (-0·21 - -0·03) | 0·007 | 79/625 | 0.32 |
| Amygdala | 753/1474 | -0·18 (-0·27 - -0·10) | $4{\cdot}90x10^{-9}$ | 80/590 | 0.41 |
| Caudate | 777/1502 | -0·10 (-0·19 - -0·01) | 0·02 | 80/627 | 0.15 |
| Hippocampus | 757/1446 | -0·08 (-0·17 - 0·003) | 0·06 | 80/593 | 0.69 |
| Pallidum | 776/1484 | 0·01 (-0·07 - 0·10) | 0·74 | 79/621 | 0.26 |
| Putamen | 784/1508 | -0·13 (-0·22 - -0·04) | 0·004 | 81/627 | 0.29 |
| Thalamus | 692/1253 | -0·03 (0·04 - -0·12) | 0·53 | 80/458 | 0.29 |
| ICV | 793/1512 | -0·06 (0·04 - -0·16) | 0·15 | 81/632 | 0.92 |

* within this group, 152 subjects were lifetime positive for the use of stimulant medication, 82 were lifetime negative; for 565 no lifetime information was available.